The Influence of Fluctuating Temperature on *Megamelus scutellaris* Berg (Hemiptera: Delphacidae)

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PURPOSE: Information from unpublished reports, field, and greenhouse observations suggests that temperatures exceeding 29° C can cause reduced development and increased mortality of *Megamelus scutellaris* Berg (waterhyacinth planthopper), a biological control agent released for the management of *Eichhornia crassipes* (Mart. Solms). This document reports the results of an experiment conducted under greenhouse conditions designed to gauge temperature impacts to *M. scutellaris* survival and reproduction under fluctuating temperatures regimes.

BACKGROUND: Waterhyacinth (*E. crassipes*) was first introduced into the U.S. in Louisiana during the International Cotton Exposition in 1884 (Center 2004). Since its initial introduction, the range of waterhyacinth has spread to include the southern and western regions of the United States and is expanding northward (U.S. Department of Agriculture/Natural Resources Conservation Service [USDA/NRCS] 2016). Waterhyacinth is capable of rapid growth and can quickly cover the water's surface thereby reducing light penetration to algae and submersed plants, lowering dissolved oxygen levels and pH, and ultimately leading to altered native species diversity (Getsinger et al. 2014; Center 2004; Villamagna and Murphy 2009). Mats of waterhyacinth have also been shown to obstruct waterways, impact irrigation and drinking water delivery, navigation, and recreation. Additionally, waterhyacinth has been documented as a human health hazard by increasing mosquito breeding habitats (Center 2004).

Four insect biological control agents have been released in the U.S. for the management of waterhyacinth (Tipping et al. 2014a; Center et al. 2004; Tipping et al. 2011) including two weevils (*Neochetina eichhorniae* Warner and *N. bruchi* Hustache) and a moth (*Niphograpta albiguttalis* Warren) (released in the 1970s), and a planthopper (*M. scutellaris*) (released in 2010). The three species released in the 1970s are well established but provide only minimal impact in many situations, especially at sites where herbicides are used on a continual basis (Center et al. 1999).

During host-specificity testing, *M. scutellaris* was shown to have a short developmental time (egg to adult in < 25 days at 25° C) and the potential to form large and damaging populations. However, despite attempts since 2010, establishment has only been reported in a limited number of areas (only in some sites in Florida and California[1]) and field impacts from the development of large and damaging populations have not been observed (Tipping et al. 2014b; Grodowitz et al. 2014;

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[1] Personal communications, Patrick Moran, 2014 and 2015, USDA, ARS.

Grodowitz et al.[1]). Reasons for lack of establishment in Louisiana (Grodowitz et al. 2014, Grodowitz et al.)[1]; some sites in Florida (Tipping et al. 2014b) and California are unknown, yet high temperatures have been identified as a possible cause. An unpublished report by South African scientists indicated that constant temperatures exceeding 29° C cause high mortality in early instars[3]. In addition, observations of survival in greenhouse colonies in Mississippi and south Louisiana indicate declines in colony populations, even to the point of total colony loss during the hotter part of the growing season (Grodowitz et al. 2014; Grodowitz et al.). Also, summer survival in outdoor colonies at the U.S. Army Engineer Lewisville Aquatic Ecology Research Facility (LAERF) near Dallas, Texas occurred only when plants were kept in water baths of about 19° C. This is in comparison to no survival without chilling, which also points to probable high temperature impacts (Grodowitz et al. [2]), especially in Dallas, where daily mean temperatures in July 2013 reached 33° C (weatherunderground 2015). In addition, establishment apparently tended to occur at sites with significant amounts of shade which may be related to lower daytime temperatures in these areas (Tipping et al. 2014b; Patrick Moran[3]).

Due to limited experimental evidence and mostly circumstantial information on probable high temperature impacts, an experiment was designed to examine temperature effects on *M. scutellaris* populations under fluctuating ambient greenhouse conditions.

MATERIALS AND METHODS: Insects used in this experiment were obtained from colonies maintained at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, Mississippi. Individuals used to initiate these colonies were obtained from several USDA-ARS sources but ultimately came from those collected during 2006 at the Otamedi Natural Preserve, Buenos Aires Province, Argentina.

Three shallow water baths (30 cm x 122 cm x 244 cm) were set to maintain water temperatures at 18° C, 25° C, and 33° C using separate Model CFF-500 Liquid Circulators from the Cornelius Remcor Products Company (Glendale Heights, Illinois). Temperatures in the water baths remained relatively constant but varied slightly with time of day. Mean water bath temperatures during the experiment were close to the intended temperatures, though min/max temperatures varied as much 9° C in the 25° C and 33° C water baths and only 4° C for the 18° C water bath (Table 1).

Table 1. Mean temperatures and associated standard error, minimum and maximum temperatures over the course of the experiment for the 18°, 25° and 33° C water baths. N = 3396.				
Water Bath	**Mean Water Bath Temperature**	**Standard Error**	**Minimum**	**Maximum**
18	16.90	0.019	15.09	19.02
25	25.14	0.031	20.33	29.35
33	33.31	0.022	27.27	36.62

[1] Grodowitz, M. J., S. Johnson, J. Woodard, N. E. Harms, G. Dick, J. Freedman, and M. Ferro. In Press. Update on the progress of rearing and establishing the Waterhyacinth Planthopper (*Megamelus scutellaris* Berg; Hemiptera: Delphacidae). ERDC/EL Technical Note. Vicksburg, MS: U.S. Army Engineer Research and Development Center.
[2] Grodowitz, M. J., S. Johnson, J. Woodard, N. E. Harms, G. Dick, J. Freedman, and M. Ferro. In Press. Update on the progress of rearing and establishing the Waterhyacinth Planthopper (*Megamelus scutellaris* Berg; Hemiptera: Delphacidae). ERDC/EL Technical Note. Vicksburg, MS: U.S. Army Engineer Research and Development Center.
[3] Personal communication, Martin Hill, April 2010, Rhodes University, South Africa.

Thirty six, 20–l plastic containers (12 containers in each water bath) were fitted with a 12.7 mm PVC down-pipe through the sidewall to allow for rapid and non-intrusive exchange of nutrient solution in the containers. A modified heavy-gauge galvanized wire tomato cage was inserted through the top rim of the container to act as support for net bags (149 μm mesh size) which covered the entire opening of the containers. The containers were filled with 12–l of a high-N (20 mg/L) nutrient solution (Table 2).

Table 2. Composition of nutrient solution used for all temperature treatments.		
Compound	Amount of Stock Solution	Concentration of Stock Solution
NH_4NO_3	21.85 ml/12 l	100 g/l
$CaCl_2$	19.94 ml/12 l	100 g/l
KNO_3	0.62 ml/12 l	100 g/l
KH_2PO_4	6.27 ml/12 l	100 g/l
$MgSO_4$	5.94 ml/12 l	250 g/l
Chelated Fe (6%)[1]	16 ml/12 l	50 g/l
Micronutrients[2]	12 ml/12 l	
$H_3BO_3*H_2O$		2.86 g/l
$MnCl_2*H_2O$		1.81 g/l
$ZnSO_4*H_2O$		0.22 g/l
$CuSO_4*H_2O$		0.8 g/l
$H_2MoO_4*H_2O$		0.17 g/l

[1] Sprint® 138 Iron Chelate Micronutrient Iron (Fe) 6%, Becker Underwood, Inc., Ames, IA

[2] All five compounds were mixed together to form one micronutrient stock solution

Reverse osmosis water was added to each container as evaporation occurred. The nutrient solution in each container was first exchanged on 16 July 2014, and then every week thereafter to limit nutrient deficiencies. On 18 June 2014, each container received one waterhyacinth plant of similar size and condition. Plants were randomly selected and placed into each container after being washed thoroughly with water and then sprayed until entirely coated with a mixture of Lambda-cyhalothrin (22.8% ai, Karate®, Syngenta, Wilmington, Delaware) at a rate of 0.12 ml/gallon of water and the adjuvant Polyalkyleneoxide (Thoroughbred®, Winfield Solutions, Saint Paul, Minnesota) at 3.5 ml/gallon of water to eliminate herbivores and other invertebrates. Containers were rotated periodically in a random fashion in each water bath.

Temperatures in each water bath and two randomly selected test containers for each treatment combination were recorded every thirty minutes by HOBO® Pendant Temperature/Light Data Loggers 64 k (Onset®, Cape Cod, Massachusetts). Pendants used in the water baths were weighted to be completely submersed while those within the containers were secured to a small sheet of closed-cell extruded polystyrene foam which allowed the pendants to float and record temperatures within the canopy approximately 4 cm above the water surface. Mean canopy temperatures based on the readings from the two pendants were used to assess canopy temperatures in each temperature regime. In addition, ambient greenhouse temperatures were recorded by hanging a pendant enclosed in a small box, with slanted opened sides to allow free air movement but minimizing solar heating, approximately 1 m above the water baths in a central greenhouse location.

The water bath system produced three temperature regimes within the canopy that fluctuated based on both water bath and ambient air temperatures (Figure 1a). Mean temperatures ($\pm$ standard error of

the mean) within the canopy at the plant base (i.e., about 4 cm from water surface) over the entire experiment were 21.61±0.055 at 18° C, 25.32±0.049 at 25° C, and 27.64±0.54 at 33° C.

While overall means for canopy temperature only varied a few degrees from each other, maximum temperatures and duration of high temperatures varied throughout the eight week experimental period. For example, canopy temperatures in the 33º C water bath replications exceeded 30° C, on average, 69% of the time during the hottest four hours of the day (i.e., between 1300 to 1700) (Figure. 1b). This is significantly more than 6% and 31% of the time above 30° C for the 18° C and 25° C water baths, respectively ($F_{(3,2528)}$ = 409.44 and $p < 0.00001$). As expected, temperatures within the canopy varied considerably but not only in relation to air temperatures. Water bath temperature was also an important contributor to modifying temperatures within the canopy. Multiple regression analysis using greenhouse air and water bath temperatures as predictors of canopy temperature exhibited a highly significant relationship and explained over 85% of the variation in canopy temperature ($F_{(2, 10,185)}$ = 30,545.95, $p < 0.00001$, $r^2 = 0.8671$) (Figure 2). This relationship is similar to what we have reported previously (Grodowitz et al. 2014).

Plants were allowed to acclimate for two weeks in their assigned temperature regimes before the addition of *M. scutellaris* individuals. Immediately prior to the addition of *M. scutellaris*, plant characteristics were recorded, including plant number, plant height, mean number of leaves per plant (determined by dividing the total number of leaves by plant number), and leaf chlorophyll. Leaf chlorophyll was measured with the atLeaf+ meter (FT Green, LLC, Wilmington, Delaware) on three randomly selected leaves per container and then averaged to obtain a single reading for each replication (Grodowitz et al. [1]). Although the atLeaf+ meter outputs unitless values, chlorophyll was determined by converting atLeaf+ meter values to soil plant analysis development (SPAD) values and subsequently to chlorophyll (μg/cm^2) based on regression equations averaged for several different plant species as given by Zhu et al. (2012):

$$Conversion\ of\ atLeaf + to\ SPAD: SPAD\ =\ 0.99\ x\ atLeaf + -10.1.$$

$$Conversions\ of\ SPAD\ to\ chlorophyll: Chlorophyll(\mu g\,/\,cm^2)\ =\ \left(SPAD\ -\ 15.1\right)/58.3.$$

On 2 July 2014, *M. scutellaris* individuals of mixed ages were randomly selected from the rearing colonies and 12 individuals were placed into each container (i.e., six replications) for each temperature treatment. On 27 and 28 August 2014, eight weeks after introducing insects onto plants, nets were removed from each container. *Megamelus scutellaris* individuals were aspirated from each container and enumerated. In addition, above-water, below-water, and dead plant biomass were quantified. In addition, number of plants, plant height, number of leaves per plant, number of flowers, and leaf chlorophyll were quantified for each replication as described previously. Above-water biomass tissues were dried in a forced-air oven at 55º C for 48 hours, weighed and then ground

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[1] Grodowitz, M. J., S. Johnson, J. Woodard, N. E. Harms, G. Dick, J. Freedman, and M. Ferro. In Press. Update on the progress of rearing and establishing the Waterhyacinth Planthopper (*Megamelus scutellaris* Berg; Hemiptera: Delphacidae). ERDC/EL Technical Note. Vicksburg, MS: U.S. Army Engineer Research and Development Center.
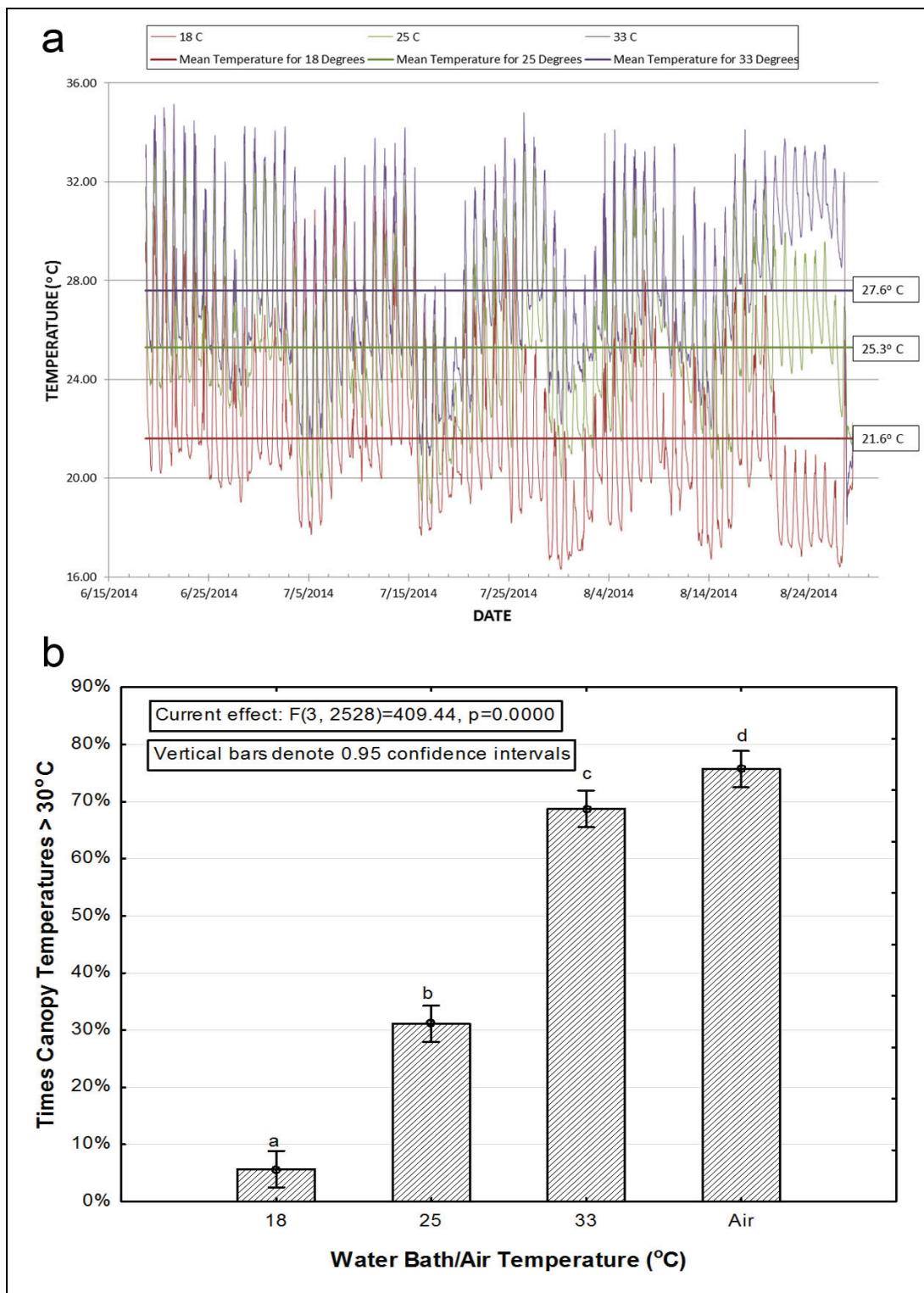
Figure 1. Temperature profiles within the canopy for each water bath temperature through the entire experimental period. Colored lines across the graph is the average canopy temperature for each water bath temperature (a) and mean percentage of times temperature exceeded 30° C within the canopy for each temperature treatment and greenhouse ambient air temperature for the hottest period of the day (i.e., 1300 to 1700; b). Means with the same letters are not significantly different at p < 0.05 level.

Canopy Temperature (°C) = 2.2813+0.496*air +0.3793*water bath
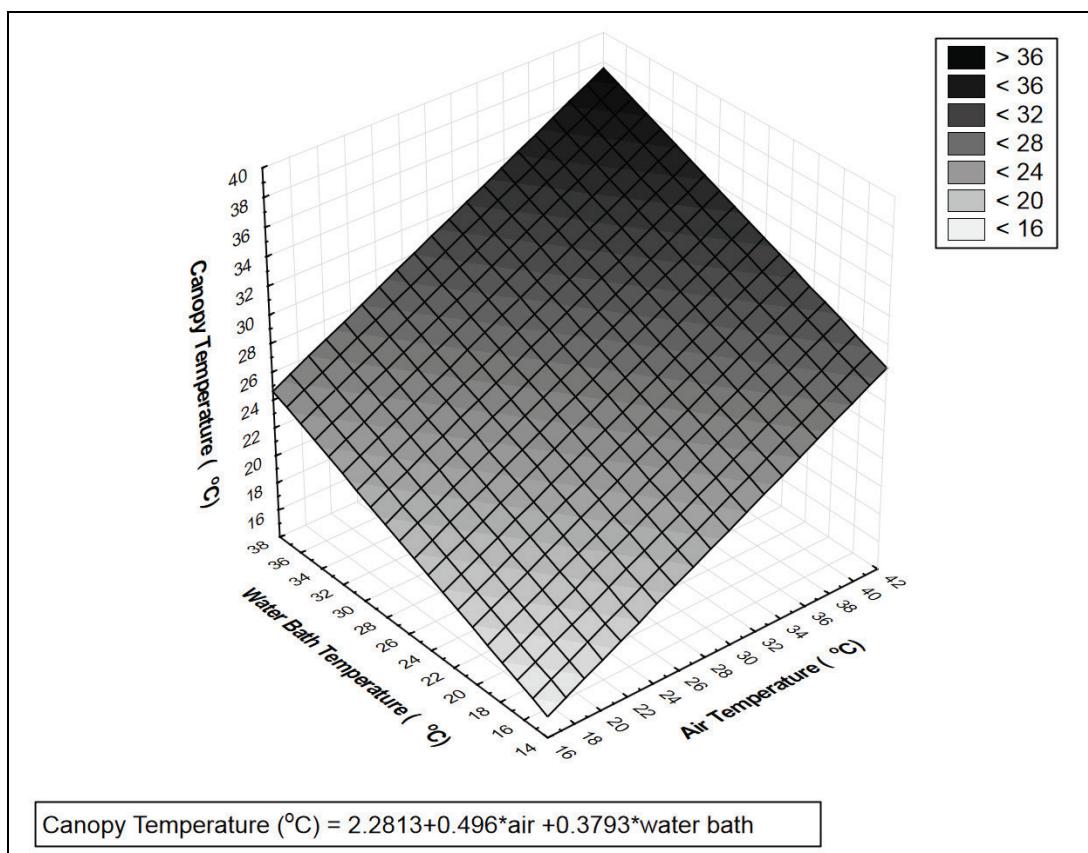
Figure 2. Multiple regression illustrating the relationship between air, water bath, and canopy temperature. Canopy temperature was measured approximately 4 cm above the water surface. The data for this regression was calculated by using all temperature readings obtained for all water baths combined.

to pass through a 0.5 mm screen. Nitrogen content was determined on a 250 mg sub-sample of each replicate, subjected to a sulfuric acid/hydrogen peroxide block digestion (Allen et al. 1974) and held at 4° C until analyzed for nitrogen content (as ammonia NH3-N) using the phenate method (American Public Health Association et al. 2012).

Statistical analyses were conducted using Statistica version 12 (Statsoft 2013). Statistical significance was determined using ANOVA and Newman-Keuls post hoc test applied to differentiate between means. The $p = 0.05$ probability level was used to determine statistical differences. Biomass is reported as dry weight (g).

RESULTS: Prior to the introduction of *M. scutellaris,* most plant characteristics were not significantly different between temperature regimes and no flowers were observed. Mean plant height ranged from 15 cm to 19 cm with no significant differences due to temperature ($F_{(2, 30)} = 1.179$, $p > 0.05$), replications destined to contain insects ($F_{(1, 30)} = 0.959$, $p > 0.05$), or the interaction between the two ($F_{(2, 30)} = 0.886$, $p > 0.05$). Similarly, no initial differences were noted for chlorophyll levels. Chlorophyll ranged from 0.4 $\mu g/cm^2$ to 0.5 $\mu g/cm^2$ with no significant differences for temperature ($F_{(2, 30)} = 1.785$, $p > 0.05$), replications destined to contain insects ($F_{(1, 30)} = 1.600$, $p > 0.05$), or the interaction between the two ($F_{(2, 30)} = 1.941$, $p > 0.05$). However, the number of plants and the number of leaves per plant were

significantly different between temperature treatments. Numbers of plants with two weeks acclimatization were significantly different with over three-fold higher number of plants observed for the 33° C treatment as compared to the 18° C treatment (Figure. 3a). As was expected, higher temperatures promoted more rapid growth (Sato 1988). The number of leaves per plant exhibited the opposite trend with about four-fold higher number of leaves per plant observed for the 18° C treatment in comparison to 33° C treatments (Figure. 3b).
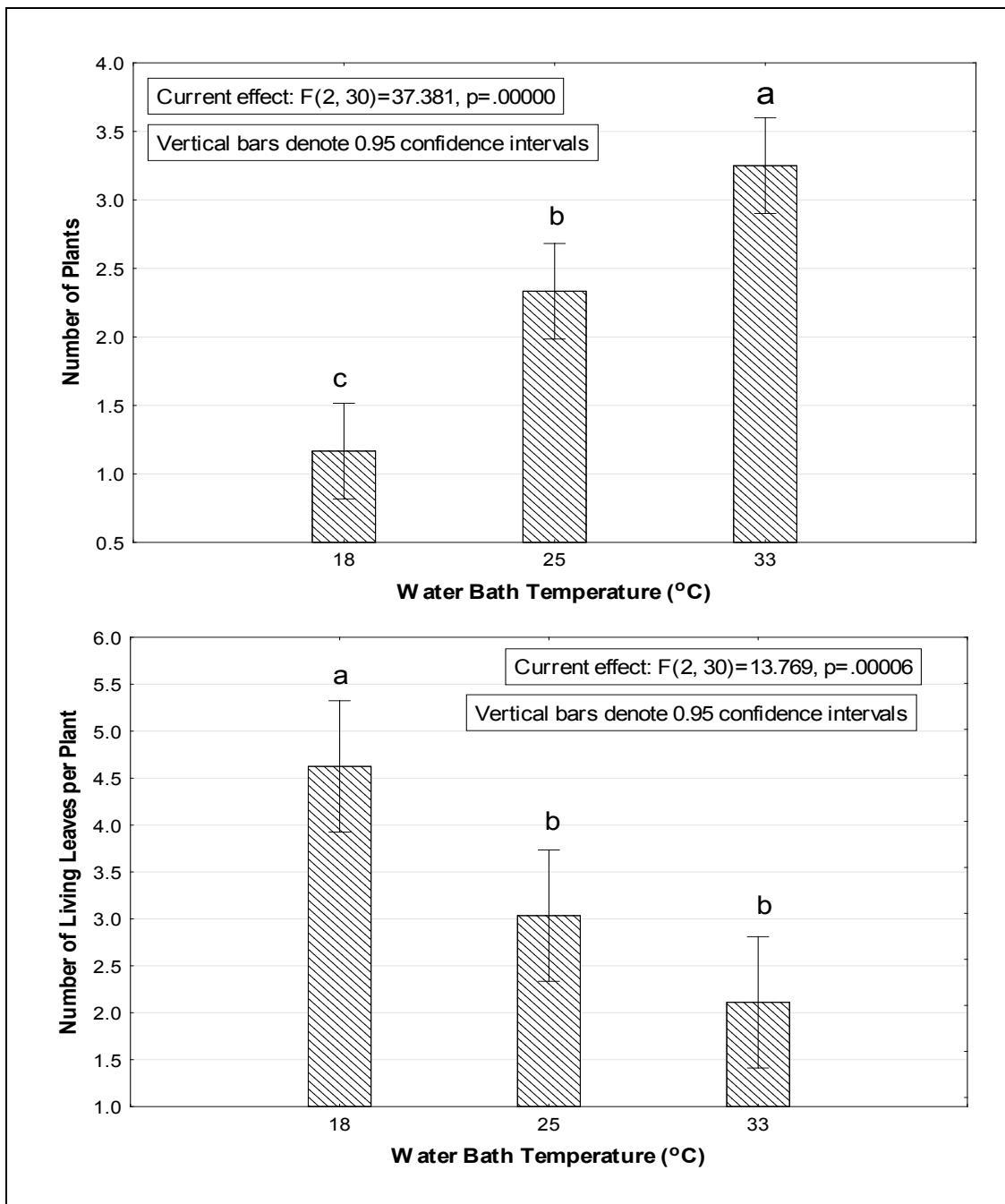
Figure 3. Mean number of plants (a) and mean number of leaves per plant (b) for all water bath temperature treatments prior to the introduction of waterhyacinth planthoppers. Means with the same letters are not significantly different at $p < 0.05$ level.

After eight weeks (at the time of harvest), above water biomass (dry weight) exhibited significant (p = 0.03) increases with rising water bath temperatures, with an almost two-fold increase in above water biomass for the 33° C water bath treatment as compared to the 18° C water bath (Figure. 4a). Above water biomass (dry weight) was also significantly impacted by the presence of *M. scutellaris* (p = 0.02) with a one-half-fold decrease in above water biomass (dry weight) for those treatments containing planthoppers (Figure. 4b). The interaction between temperature and insect presence was not significant (p > 0.05). The number of living leaves also exhibited significance (p = 0.017) between temperature treatments with 1.7-fold higher number of living leaves present in the 33° C water bath compared to the 18° C treatment (Figure 5). The number of living leaves per plant was not significantly affected by insect presence or the interaction between temperature and insect presence (p > 0.05).

Predicted leaf chlorophyll (Figure. 6a) and leaf nitrogen (Figure. 6b) both exhibited decreasing trends with increasing water bath temperatures. Reductions in chlorophyll and nitrogen between the 18° C and 33° C temperature treatments were about 70% and 80% lower, respectively. Decreases in chlorophyll and nitrogen levels with increasing temperature are most likely related to increased biomass production at higher temperatures thus spreading the available nitrogen across larger quantities of biomass. Insect presence did not influence chlorophyll or nitrogen, nor was the interaction between insect presence and temperature significant (p > 0.05).

Numbers of *M. scutellaris* individuals were influenced by temperature. The temperature treatment was significant (p < 0.05) with the highest number found for the 25° C treatment with over 150 individuals on average (Figure. 7). This is in comparison to the 18° C and 33° C treatments that contained three- and fifteen-fold lower numbers of *M scutellaris* individuals after eight weeks, respectively.

DISCUSSION: High and low temperatures have a strong negative influence on the survival and reproduction of *M. scutellaris*. Significantly lower numbers of individuals were found in the current experiment when reared at either the 18° C or 33° C regime compared to the optimum 25° C. This follows information provided in an un-published report produced by South African researchers[1] who reported optimum survival at 25° C, less than 25% survival at 19° C and no survival of immatures when subjected to constant temperatures >29° C[2]. While lower survival and reproduction was observed in our 33° C treatment in comparison to 25° C, survival and reproduction did occur; planthopper numbers increased nearly nine-fold in eight weeks. Survival and reproduction at the highest temperature treatment is apparently due to a fluctuating temperature regime in which planthoppers were exposed to consecutive temperatures >29° C for typically only a few hours each day (Figure. 8a, b). For the 33° C temperature regime, the number of consecutive hours of > 30° C ranged from a low of approximately 5.5 hours to a maximum of 24 hours, though the latter only occurred two days in late August. Mean consecutive hours above 30° C were nearly six in June, five in July, and more than eight in August. Temperatures greater than ≥30° C were common in the 33° C temperature regime; it exceeded this limit nearly 70% of the time during the hottest part of the day (Figure. 2) and only 30% of the time at 25° C. The fluctuating temperatures in this experiment better reflect the real-world conditions planthoppers are exposed to upon release in the field and provide an enhanced understanding of temperature impacts and potential for establishment.

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[1] Personal communication, Martin Hill, April 2010, Rhodes University, South Africa.
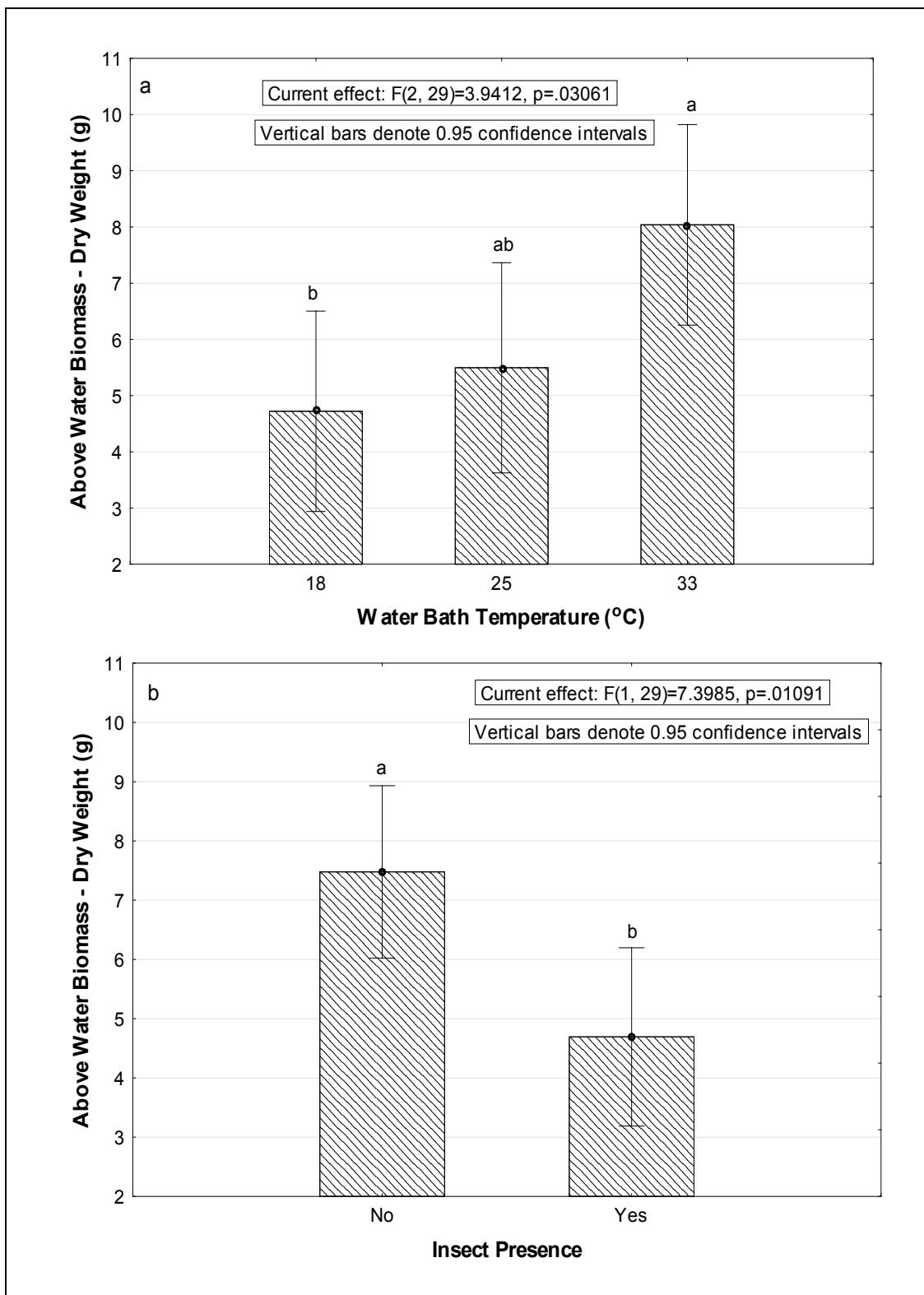
Figure 4. Mean above water biomass dry weight (g) in relation to water bath temperatures (a) and presence or absence of M. scutellaris (b). Means with the same letters are not significantly different at p < 0.05 level.
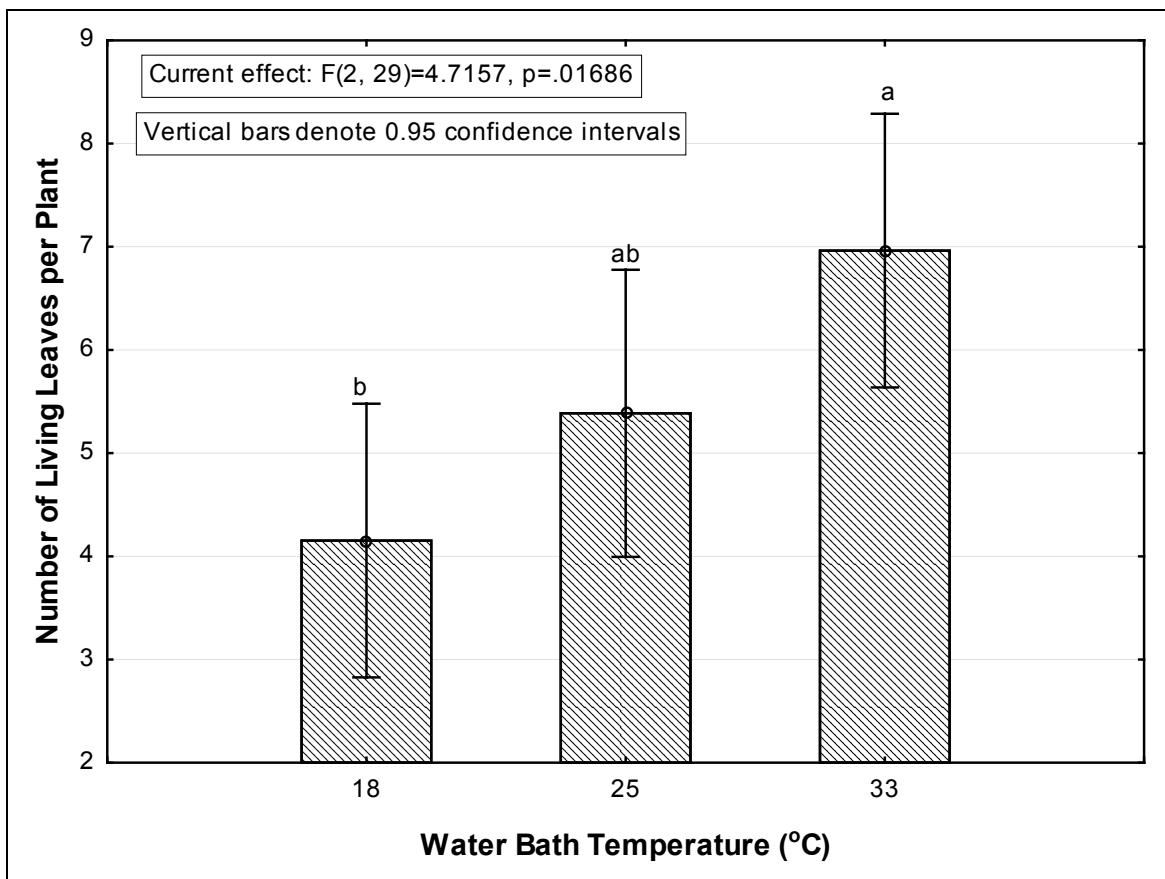
Figure 5.   Mean number of living leaves per plant for each water bath temperature. Means with the same letters are not significantly different at p < 0.05 level.

These data also illustrate the importance of water temperature, not only air temperature, in predicting establishment success based on high temperature limits. Since temperature within the canopy is moderated by air and water temperatures, establishment is possible even in areas where air temperatures may often exceed 30° C, but only when water temperatures remain low. For example, using the equation from Figure 1 to predict canopy temperature; when air temperature and water temperature are 30° C, temperatures within the canopy will approach 30° C. Lowering the hypothetical water temperature to 25° C decreases the predicted canopy temperature three degrees to 27° C. A further decrease in water temperature to 22° C further leads to a lowered predicted canopy temperature of 26° C. In contrast, early season releases of planthoppers should be timed so that they are not exposed to prolonged low temperatures.
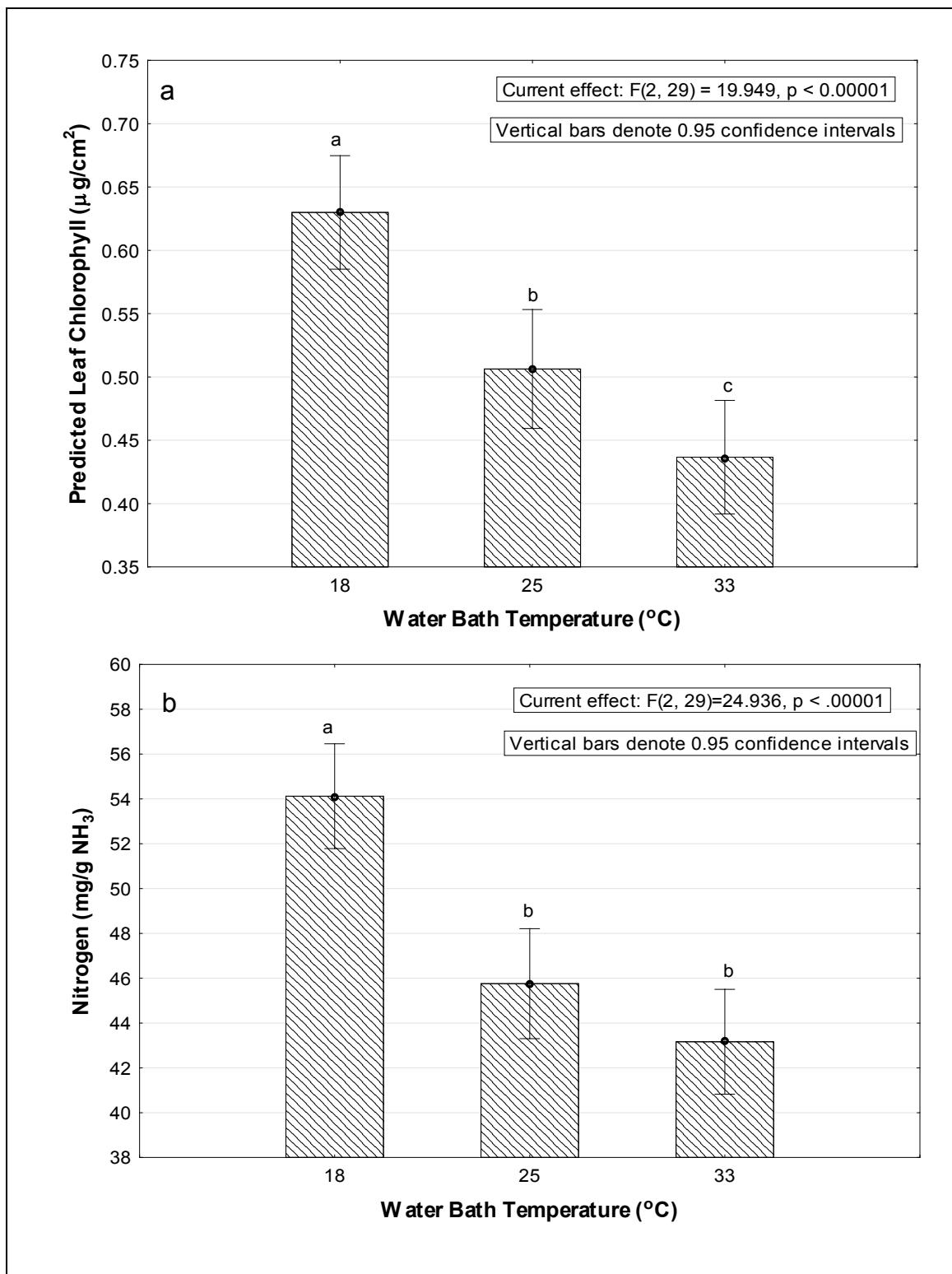
Figure 6.   Mean predicted leaf chlorophyll (a) and mean nitrogen levels (b) across all three temperature treatments. Means with the same letters are not significantly different at p < 0.05 level.
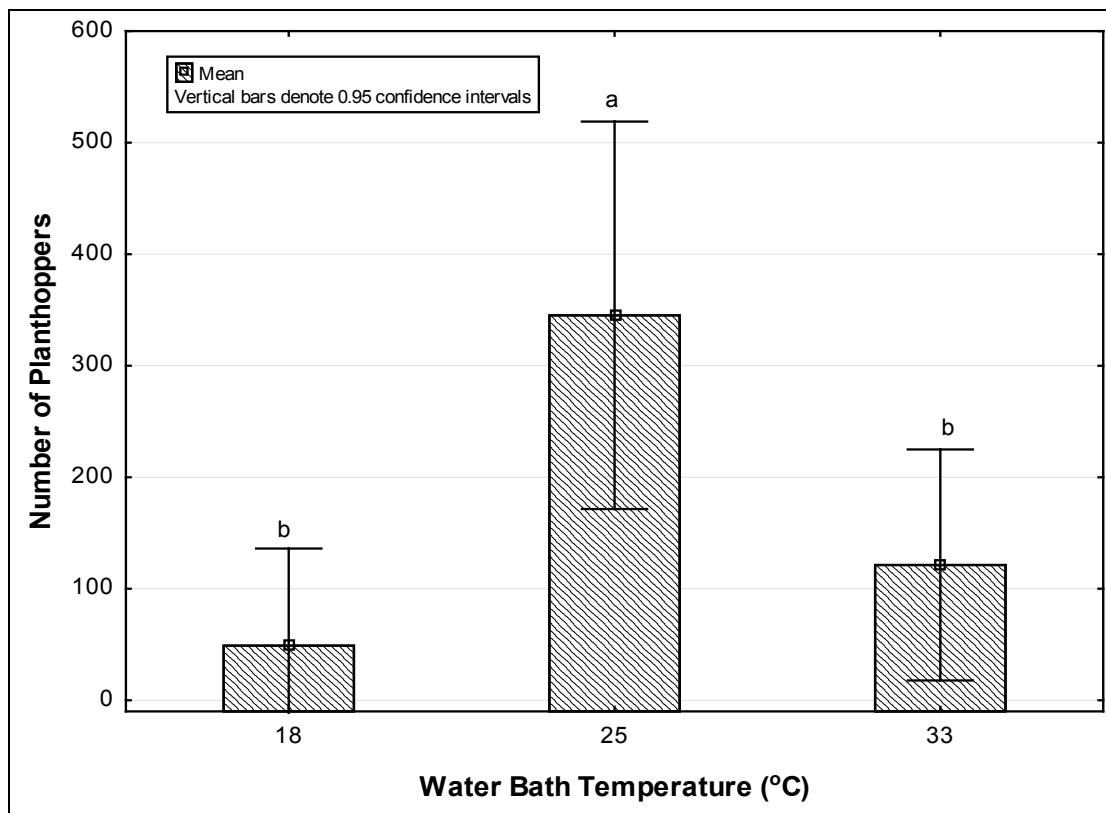
Figure 7. Mean number of planthoppers in relation to water bath temperatures. Means with the same letter are not significantly different at $F_{(2, 14)} = 5.931$, $p = 0.0136$.

Importantly, the waterhyacinth planthopper exhibits thermoregulatory behaviors in response to high temperatures (Grodowitz, personal observation). It has been observed that individuals move up and down within the canopy depending on temperature (i.e., higher numbers of individuals remain close to the water surface during the hotter part of the day). This is especially relevant since temperatures in the upper canopy are almost exclusively moderated by ambient air temperatures (Grodowitz et al. 2014). We have also observed higher numbers of planthoppers in our rearing colony on plants nearest the greenhouse cooling pads. Thermoregulatory behavior enables *M. scutellaris* to avoid exposure to high lethal temperatures by locating areas within the canopy exhibiting lowered temperatures thereby increasing the probability of decreased mortality due to high temperature effects. Thermoregulatory behavior is common in insects and similar behaviors have been observed in other insect species (Whitman 1987; Heinrich 1993).

Apparently, changes in plant characteristics did not play a role in *M. scutellaris* survival. We did not observe any statistically significant effects to planthopper numbers due to changes in waterhyacinth growth characteristics or nitrogen level. However, nitrogen levels in this experiment for all temperature regimes were well within expected optimum levels for waterhyacinth growth and presumably for adequate planthopper development and survival. Plant tissue nitrogen levels for any given replication never fell below 4%. However, more research is needed to examine the importance of plant nutritional quality on planthopper growth and development as well as the interaction between nutrition and temperature. Studies have indicated that temperature impacts on insects may be altered based on plant nutritional status (Room et al. 1989).
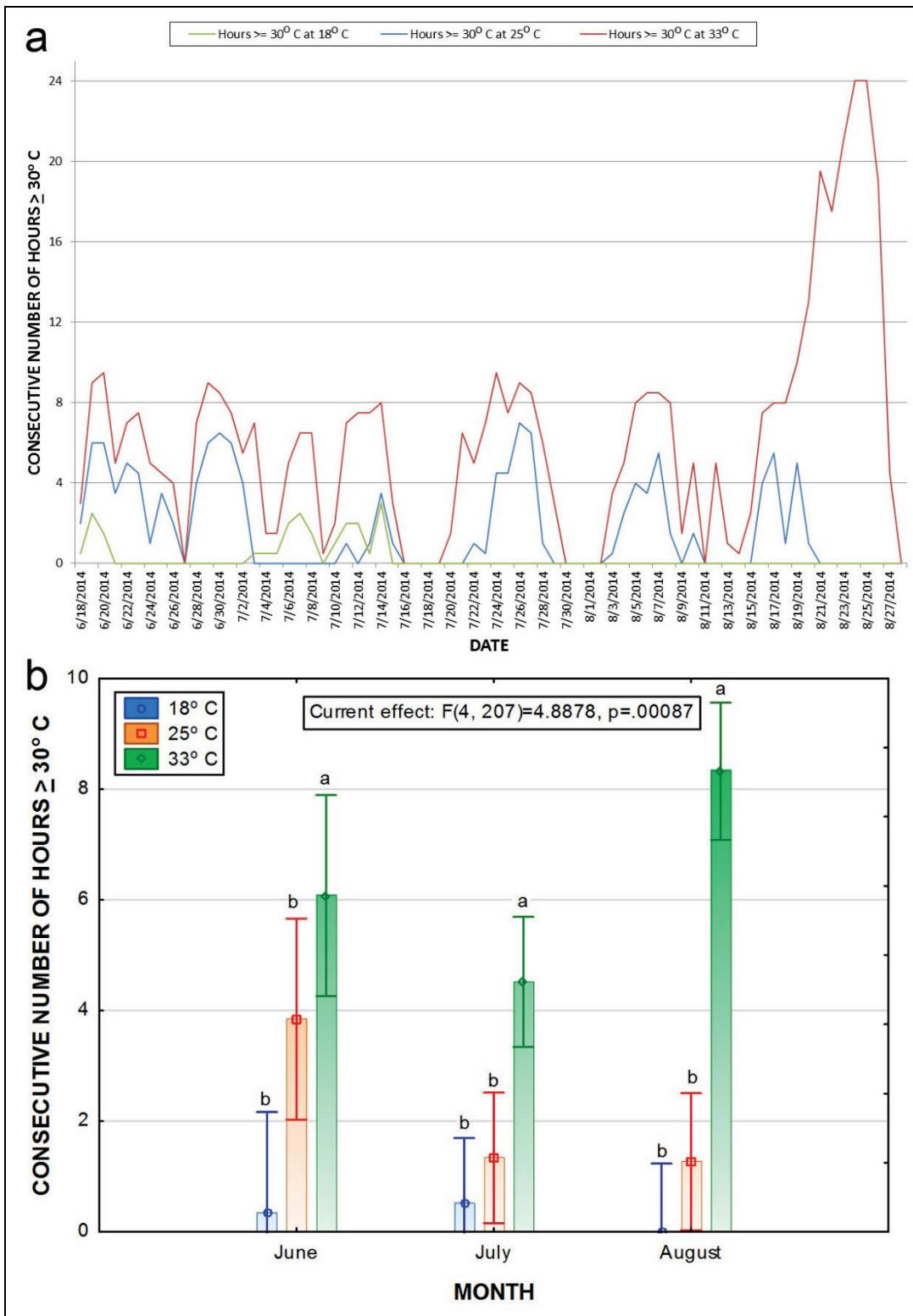
Figure 8. Consecutive number of hours temperatures each day exceed or equal 30° C for each temperature regime over the course of the experiment (a) and consecutive mean number of hours monthly temperatures exceed or equal to 30° C for each temperature regime (b). Means with the same letter within a specific month are not significantly different at $p < 0.05$. Vertical bars represent the 0.95 confidence intervals.

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